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RESEARCH ARTICLE

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New sections in *Penicillium* containing novel species producing patulin, pyripyropens or other bioactive compounds

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Key words

Aspergillaceae
extrolites
food spoilage
phylogeny
taxonomy

Abstract Subgenera and sections have traditionally been used in *Penicillium* classifications. In the past, this sectional classification was based on macro- and microscopic characters, and occasionally supplemented with physiological and/or extrolite data. Currently, 25 sections are accepted, largely based on phylogenetic data. Certain sections of subgenus *Penicillium* were never studied in detail using a multigene sequence approach combined with phenotypic, ecological and extrolite data. Based on a combined partial β-tubulin (*BenA*), calmodulin (*CaM*) and RNA polymerase II second largest subunit (*RPB2*) multigene sequence dataset, we introduce two new sections (*Osmophila* and *Robsamsonia*) in subgenus *Penicillium* and synonymize section *Digitata* with section *Penicillium*. The phylogeny correlates well with phenotypic, physiological and ecological data, and some extrolites were diagnostic for certain *Penicillium* sections. Furthermore, four new species belonging to the newly introduced sections are described using a polyphasic approach, including *BenA*, *CaM* and *RPB2* sequences, macro- and micromorphological data and extrolite profiles. The new section *Robsamsonia* and the new species *Penicillium robsamsonii* and *Penicillium samsonianum* were introduced to celebrate Dr. Robert A. Samson's 70th birthday.

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INTRODUCTION

Dierckx (1901) first proposed an infrageneric classification system in *Penicillium*. In the major *Penicillium* monographs published later, various subgenera, sections, subsections and series were employed. Most of these infrageneric classifications were based on conidiophore branching patterns, growth rates on agar media, extrolite data and/or physiological features (Biourge 1923, Raper & Thom 1949, Pitt 1980, Ramirez 1982, Stolk & Samson 1985, Frisvad & Samson 2004). Based on a four-gene phylogeny, Houbraken & Samson (2011) subdivided the genus into two subgenera and 25 sections. For the species traditionally classified in subgenus *Penicillium*, they followed Frisvad & Samson's (2004) sectional classification and grouped the species in concordance with that publication. However, based on a *RPB2* phylogeny, it was clear that certain species, including *P. osmophilum*, *P. coprophilum* and *P. coprobium*, could not be placed reliably in known sections (Houbraken & Samson 2011).

Coprophilic fungi, including Penicillia, inhabit a competitive substrate with many micro-organisms and may benefit if they are able to produce bioactive compounds (Frisvad et al. 2004, Bills et al. 2013). Based on similarities in ecology, morphology and extrolites, most of the coprophilic Penicillia were classified in series *Claviformia*: *P. brevistipitatum*, *P. clavigerum*, *P. concentricum*, *P. coprobium*, *P. coprophilum*, *P. formosanum*, *P. glandicola* and *P. vulpinum* (Frisvad & Samson 2004, Wang & Zhuang 2005). These coprophilic species and species in the series *Expansa* and *Urticicola* nearly all produce patulin, which is both a mycotoxin and an antibiotic (Frisvad & Samson 2004, Frisvad et al. 2004, Dombrinck-Kurtzman & McGovern 2007). A phylogenetic analysis of the patulin-producing Penicillia based on the isoepoxydon dehydrogenase (*idh*) gene and rDNA sequences (Dombrinck-Kurtzman 2007) was incongruent with household gene cladification and this was further supported by the analyses of Houbraken & Samson (2011). These data indicate that the phylogenetic relationships of patulin-producing *Penicillium* need further study and a new more in-depth phylogenetic analysis of these species is needed.

Discovery of new taxa will help to provide a more robust phylogeny (Graybeal 1998), and in this paper we describe four new related species that will help place the coprophilic Penicillia in a more accurate phylogenetic context. Firstly, we re-evaluated the classification of sections *Fasciculata*, *Digitata*, *Penicillium*, *Roquefortorum* and *Chrysogena* as delimited by Frisvad & Samson (2004). From our analysis of a three-gene phylogeny of partial β-tubulin (*BenA*), calmodulin (*CaM*) and RNA polymerase II second largest subunit (*RPB2*) sequences, we propose two new sections, and this classification correlated well with phenotypic, physiological and ecological data. Secondly, the novel species belonging to the newly introduced sections were studied using a combination of phenotypic characters, extrolite patterns and sequence data (*BenA*, *CaM*, *RPB2*).

MATERIAL AND METHODS

Strains

Strains used in the multigene phylogeny were mainly obtained from the culture collection of the CBS-KNAW Fungal Biodiversity Centre in the Netherlands (CBS) (Table 1). The new species described in this study were isolated during different surveys and maintained in three different culture collections: CBS, the China General Microbiological Culture Collection in the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (CGMCC) and the culture collection of DTU Systems Biology, Lyngby, Denmark (IBT).

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Table 1 Overview of species and strains used in Fig. 1 and their current and previous section designation. The strain numbers and the corresponding *BenA*, *CaM* and *RPB2* GenBank numbers used to generate Fig. 1 are included.

Designation clade (Fig. 1)	Species name	Current sectional classification	Sectional classification acc. Houbraken & Samson 2011	Strain	GenBank no.		
					BenA	*CaM*	*RPB2*
Clade 1	*Penicillium albocoremium*	*Fasciculata*	*Fasciculata*	CBS 472.84	KU896812	KU896819	KU904344
Clade 1	*P. allii*	*Fasciculata*	*Fasciculata*	CBS 131.89	AY674331	KU896820	KU904345
Clade 1	*P. aurantiogriseum*	*Fasciculata*	*Fasciculata*	CBS 324.89	AY674296	KU896822	JN406573
Clade 1	*P. biforme*	*Fasciculata*	–	CBS 297.48	FJ930944	KU896823	KU904346
Clade 1	*P. camemberti*	*Fasciculata*	*Fasciculata*	MUCL 29790	FJ930956	KU896825	JN121484
Clade 1	*P. caseifulvum*	*Fasciculata*	*Fasciculata*	CBS 101134	AY674372	KU896826	KU904347
Clade 1	*P. cavernicola*	*Fasciculata*	*Fasciculata*	CBS 100540	KJ834439	KU896827	KU904348
Clade 1	*P. commune*	*Fasciculata*	*Fasciculata*	NRRL 890	AY674366	KU896829 (CBS 311.48)	KU904350 (CBS 122424)
Clade 1	*P. crustosum**	*Fasciculata*	*Fasciculata*	n/a	n/a	n/a	n/a
Clade 1	*P. cyclopium*	*Fasciculata*	*Fasciculata*	CBS 144.45	AY674310	KU896832	JN985388
Clade 1	*P. discolor*	*Fasciculata*	*Fasciculata*	CBS 474.84	AY674348	KU896834	KU904351
Clade 1	*P. echinulatum*	*Fasciculata*	*Fasciculata*	CBS 317.48	AY674341	DQ911133 (ATCC 10434)	KU904352
Clade 1	*P. freii*	*Fasciculata*	*Fasciculata*	CBS 476.84	KU896813	KU896836	KU904353
Clade 1	*P. gladioli*	*Fasciculata*	*Penicillium*	CBS 332.48	AY674287	KU896837	JN406567
Clade 1	*P. hirsutum*	*Fasciculata*	*Fasciculata*	CBS 135.41	AF003243	KU896840	JN406629
Clade 1	*P. hordei*	*Fasciculata*	*Fasciculata*	CBS 701.68	AY674347	KU896841	KU904355
Clade 1	*P. melanoconidium*	*Fasciculata*	*Fasciculata*	CBS 115506	AY674304	KU896843	KU904358
Clade 1	*P. neoechinulatum*	*Fasciculata*	*Fasciculata*	CBS 101135	AF003237	KU896844	JN985406
Clade 1	*P. nordicum*	*Fasciculata*	*Fasciculata*	DTO 098-F7	KJ834476	KU896845	KU904359
Clade 1	*P. palitans*	*Fasciculata*	*Fasciculata*	CBS 107.11	KJ834480	KU896847	KU904360
Clade 1	*P. polonicum*	*Fasciculata*	*Fasciculata*	CBS 222.28	AY674305	KU896848	JN406609
Clade 1	*P. radicicola**	*Fasciculata*	*Fasciculata*	n/a	n/a	n/a	n/a
Clade 1	*P. solitum*	*Fasciculata*	*Fasciculata*	CBS 424.89	AY674354	KU896851	KU904363
Clade 1	*P. thymicola*	*Fasciculata*	*Fasciculata*	CBS 111225	AY674321	FJ530990	KU904364
Clade 1	*P. tricolor*	*Fasciculata*	*Fasciculata*	CBS 635.93	AY674313	KU896852	JN985422
Clade 1	*P. tulipae**	*Fasciculata*	*Fasciculata*	n/a	n/a	n/a	n/a
Clade 1	*P. venetum*	*Fasciculata*	*Fasciculata*	CBS 201.57	AY674335	KU896855	KU904366
Clade 1	*P. verrucosum*	*Fasciculata*	*Fasciculata*	CBS 603.74	AY674323	DQ911138 (IMI 200310)	JN121539
Clade 1	*P. viridicatum*	*Fasciculata*	*Fasciculata*	CBS 390.48	AY674295	KU896856	JN121511
Clade 2	*P. clavigerum*	*Penicillium*	*Penicillium*	CBS 255.94	AY674427	KU896828	KU904349
Clade 2	*P. coccotrypicola**	*Penicillium*	*Penicillium*	n/a	n/a	n/a	n/a
Clade 2	*P. digitatum*	*Penicillium*	*Digitata*	CBS 112082	KJ834447	KU896833	JN121426
Clade 2	*P. expansum*	*Penicillium*	*Penicillium*	CBS 325.48	AY674400	DQ911134	JF417427
Clade 2	*P. italicum*	*Penicillium*	*Penicillium*	CBS 339.48	AY674398	DQ911135	JN121496
Clade 2	*P. marinum*	*Penicillium*	*Penicillium*	CBS 109550	AY674392	KU896842	KU904357
Clade 2	*P. sclerotigenum*	*Penicillium*	*Penicillium*	CBS 101033	AY674393	KU896850	JN406652
Clade 2	*P. ulaiense*	*Penicillium*	*Penicillium*	CBS 210.92	AY674408	KU896854	KU904365
Clade 3	*P. allii-sativi*	*Chrysogena*	–	CBS 132074	JX996891	JX996232	JX996627
Clade 3	*P. chrysogenum*	*Chrysogena*	*Chrysogena*	CBS 306.48	JF909955	JX996273	JN121487
Clade 3	*P. confertum*	*Chrysogena*	*Chrysogena*	CBS 171.87	AY674373	JX996963	JX996708
Clade 3	*P. desertorum*	*Chrysogena*	–	CBS 131543	JX996818	JX996937	JX996682
Clade 3	*P. dipodomyis*	*Chrysogena*	*Chrysogena*	CBS 110412	AY495991	JX996950	JF909932
Clade 3	*P. egyptiacum*	*Chrysogena*	*Chrysogena*	CBS 244.32	KU896810	JX996969	JN406598
Clade 3	*P. flavigenum*	*Chrysogena*	*Chrysogena*	CBS 419.89	AY495993	JX996281	JN406551
Clade 3	*P. glycyrrhizacola*	*Chrysogena*	–	G4432	KF021538	KU896839 (CBS 140376)	KF021554
Clade 3	*P. goetzii*	*Chrysogena*	–	CBS 285.73	KU896815	JX996971	JX996716
Clade 3	*P. halotolerans*	*Chrysogena*	–	CBS 131537	JX996816	JX996935	JX996680
Clade 3	*P. kewense*	*Chrysogena*	*Chrysogena*	CBS 344.61	KU896816	JX996973	JF417428
Clade 3	*P. lanosocoeruleum*	*Chrysogena*	*Chrysogena*	CBS 484.84	KU896817	JX996967	JX996723
Clade 3	*P. mononematosum*	*Chrysogena*	*Chrysogena*	CBS 172.87	AY495997	JX996964	JX996709
Clade 3	*P. nalgiovense*	*Chrysogena*	*Chrysogena*	CBS 352.48	KU896811	JX996974	JX996719
Clade 3	*P. persicinum*	*Chrysogena*	*Chrysogena*	CBS 111235	JF909951	JX996954	JN406644
Clade 3	*P. rubens*	*Chrysogena*	*Chrysogena*	CBS 129667	JF909949	JX996263	JX996658
Clade 3	*P. sinaicum*	*Chrysogena*	*Chrysogena*	CBS 279.82	KU896818	JX996970	JN406587
Clade 3	*P. tardochrysogenum*	*Chrysogena*	–	CBS 132200	JX996898	JX996239	JX996634
Clade 3	*P. vanluykii*	*Chrysogena*	–	CBS 131539	JX996879	JX996220	JX996615
Clade 4	*P. osmophilum*	*Osmophila*	*Fasciculata*	CBS 462.72	AY674376	KU896846	JN121518
Clade 4	*P. samsonianum*	*Osmophila*	–	AS3.15403	KJ668582	KJ668586	KT698899
Clade 4	*P. samsonianum*	*Osmophila*	–	CBS 131220	KT698883	KT698892	KT698902
Clade 4	*P. samsonianum*	*Osmophila*	–	CBS 316.97	KT698881	KT698890	KT698900
Clade 4	*P. samsonianum*	*Osmophila*	–	CBS 343.61	KT698884	KT698893	KT698903
Clade 4	*P. samsonianum*	*Osmophila*	–	CBS 512.73	KT698882	KT698891	KT698901
Clade 5	*P. carneum*	*Roquefortorum*	*Roquefortorum*	CBS 112297	AY674386	HQ442322	JN406642
Clade 5	*P. paneum*	*Roquefortorum*	*Roquefortorum*	CBS 101032	AY674387	HQ442331	KU904361
Clade 5	*P. psychrosexualis*	*Roquefortorum*	*Roquefortorum*	CBS 128137	HQ442356	HQ442330	KU904362
Clade 5	*P. roqueforti*	*Roquefortorum*	*Roquefortorum*	CBS 221.30	AF000303	HQ442332	JN406611
Clade 6	*P. brevistipitatum*	*Robsamsonia*	*Penicillium*	AS 3.6887	DQ221695	KU896824 (CBS 122277)	JN406528 (CBS 122277)
Clade 6	*P. compactum*	*Robsamsonia*	–	AS3.15411	KM973203	KM973200	KT698909
Clade 6	*P. concentricum*	*Robsamsonia*	*Penicillium*	CBS 477.75	AY674413	DQ911131	KT900575
Clade 6	*P. coprobium*	*Robsamsonia*	*Penicillium*	CBS 561.90	AY674425	KU896830	KT900576
Clade 6	*P. coprophilum*	*Robsamsonia*	*Penicillium*	CBS 110760	AY674421	KU896831	JN406645
Clade 6	*P. dipodomyicola*	*Robsamsonia*	*Penicillium*	CBS 173.87	AY674409	KT900573	KT900577
Clade 6	*P. fimorum*	*Robsamsonia*	–	CBS 140575	KT698889	KT698898	KT698908

Table 1 (cont.)

Designation clade (Fig. 1)	Species name	Current sectional classification	Sectional classification acc. Houbraken & Samson 2011	Strain	GenBank no. *BenA*	GenBank no. *CaM*	GenBank no. *RPB2*
Clade 6	*P. fimorum*	*Robsamsonia*	–	CBS 140576	KT698888	KT698897	KT698907
Clade 6	*P. fimorum*	*Robsamsonia*	–	DTO 159-F1	KT698889	KT698898	KT698908
Clade 6	*P. glandicola*	*Robsamsonia*	*Penicillium*	CBS 498.75	KU896814	KU896838	KU904354
Clade 6	*P. griseofulvum*	*Robsamsonia*	*Penicillium*	CBS 185.27	JF909942	KT900574	JN121449
Clade 6	*P. robsamsonii*	*Robsamsonia*	–	CBS 140573	KT698885	KT698894	KT698904
Clade 6	*P. robsamsonii*	*Robsamsonia*	–	CBS 140574	KT698886	KT698895	KT698905
Clade 6	*P. vulpinum*	*Robsamsonia*	*Penicillium*	CBS 126.23	KJ834501	KU896857	KU904367
Basal group	*P. brevicompactum*	*Brevicompacta*	*Brevicompacta*	CBS 257.29	AY674437	AY484813 (NRRL 864)	JN406594
Basal group	*P. buchwaldii*	*Brevicompacta*	*Brevicompacta*	CBS 117181	JX313182	JX313148	JN406637
Basal group	*P. olsonii*	*Brevicompacta*	*Brevicompacta*	CBS 232.60	AY674445	DQ658165 (NRRL 13058)	JN121464
Basal group	*P. spathulatum*	*Brevicompacta*	*Brevicompacta*	CBS 117192	JX313183	JX313149	JN406636
Basal group	*P. tularense*	*Brevicompacta*	*Brevicompacta*	AS 3.14006	KC427175	JX313135 (CBS 430.69)	JN121516 (CBS 430.69)
Basal group	*P. canescens*	*Canescentia*	*Canescentia*	CBS 300.48	JX140946	AY484810 (NRRL 910)	JN121485
Basal group	*P. sacculum*	*Eladia*	*Eladia*	CBS 231.61	KJ834488	KU896849	JN121462
Basal group	*P. malodoratum*	*Paradoxa*	*Paradoxa*	NRRL 5083	EF669681	FJ530972 (CBS 490.64)	EF669672
Basal group	*P. paradoxum*	*Paradoxa*	*Paradoxa*	NRRL 2162	EF669683	EF669692	EF669670
Basal group	*P. lanosum*	*Ramosa*	*Ramosa*	NRRL 2009	DQ285627	FJ530974 (CBS 106.11)	KU904356 (CBS 106.11)
Basal group	*P. madriti*	*Ramosa*	*Ramosa*	CBS 347.61	KJ834470	EU644076 (IMI 86563)	JN406561
Basal group	*P. swiecickii*	*Ramosa*	*Ramosa*	CBS 119391	KJ834494	KJ866993	JN406635
Basal group	*P. atramentosum*	*Turbata*	*Turbata*	CBS 291.48	AY674402	KU896821	JN406584
Basal group	*P. turbatum*	*Turbata*	*Turbata*	CBS 237.60	KJ834499	KU896853 (CBS 383.48)	JN406556 (CBS 383.48)
Basal group	*P. formosanum*	Undefined, new section	*Penicillium*	CBS 211.92	AY674426	KU896835	JN406615

* Species names marked with an asterisk are not included in Fig. 1, but are included in this Table in order to give a complete overview of species belonging to each section.

Morphological studies

Colony characters were documented on Czapek yeast autolysate agar (CYA), CYA supplemented with 5 % NaCl (CYAS), creatine sucrose agar (CREA), dichloran 18 % glycerol agar (DG18), malt extract agar (MEA; Oxoid) and yeast extract sucrose agar (YES). Growth was also measured on CYA incubated at 15, 30 and 37 °C (referred to as CYA15°C, CYA30°C and CYA37°C, respectively). All media were prepared, inoculated and incubated following the methods of Visagie et al. (2014). Examination of the cultures growing on MEA at 25 °C was performed as described by Houbraken et al. (2014). The production of alkaloids reacting with the Ehrlich reagent was examined using a filter paper method (Lund 1995). The appearance of a violet ring after 10 minutes was considered as a positive reaction; all other colours were considered as a negative reaction.

DNA extraction, PCR and sequencing

DNA extraction was performed as described by Scott et al. (2000). Partial β-tubulin gene (*BenA*) sequences were amplified using the sense primers I2 (Wang & Wang 2013) or Bt2a, with the antisense primer Bt2b (Glass & Donaldson 1995); the ITS1-5.8S-ITS2 region of rDNA was amplified using the primer combinations ITS5/ITS4 or V9G/LS266 (White et al. 1990, Gerrits van den Ende & De Hoog 1999); the calmodulin gene (*CaM*) was amplified using the primers described by Wang (2012). A part of the *RPB2* gene was amplified using the primers RPB2-5F_Eur and RPB2-7CR_Eur (Houbraken et al. 2012b). PCR, sequencing and sequence annotation was carried out according the method described by Houbraken et al. (2012b). Newly generated sequences were deposited in GenBank (see Fig. 2 and Table 1).

Phylogenetic analysis

All datasets were aligned using the Muscle software incorporated in the MEGA v. 6 package (Tamura et al. 2013). The sections in subgenus *Penicillium* were delimited using a combined dataset of *BenA*, *CaM* and *RPB2* sequences. The newly generated sequences were supplemented with a selection of validated *Penicillium* subgenus *Penicillium* sequences (Visagie et al. 2014). An overview of strains and sequences used to study the sectional relationship are summarised in Table 1. The phylogeny of the new species together with their close relatives was studied by comparing single gene and combined phylogenies. The combined dataset was analysed by maximum likelihood analysis (ML) using the RAxML (randomised accelerated maximum likelihood) software (Stamatakis et al. 2008) and Bayesian tree inference (BI) analysis was performed using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). The Bayesian analysis was performed as previously described (Houbraken et al. 2014). The single gene phylogenies were analysed using ML analyses in MEGA v. 6 (Tamura et al. 2013). The best model for ML was selected based on the Akaike Information Criterion (AIC), calculated in MEGA. Support in nodes was calculated using a bootstrap analysis of 1 000 replicates. *Talaromyces flavus* CBS 310.38^{T} was used as outgroup in the investigation of the sectional classification. *Penicillium brevicompactum* CBS 257.29^{T} (*BenA*, *RPB2*) and NRRL 864^{T} (*CaM*) were used in the phylogenetic analysis of the relationship of the new species and alignments and trees are deposited in TreeBASE under number 19151.

Extrolite analysis

Culture extracts were made from fungal cultures grown on CYA and YES for 7–10 d at 25 °C. Extracts were prepared and analysed using the protocols summarised by Yilmaz et al. (2014). Extrolite standards have been collected either from commercial sources, as gifts from other research groups, or purified from projects and used as a library to identify the compounds produced by the *Penicillium* species investigated in this study (Klitgaard et al. 2014).

RESULTS AND DISCUSSION

Phylogeny

In total, 93 mostly ex-(neo)type strains were included in the analysis of the combined dataset and the total length of the

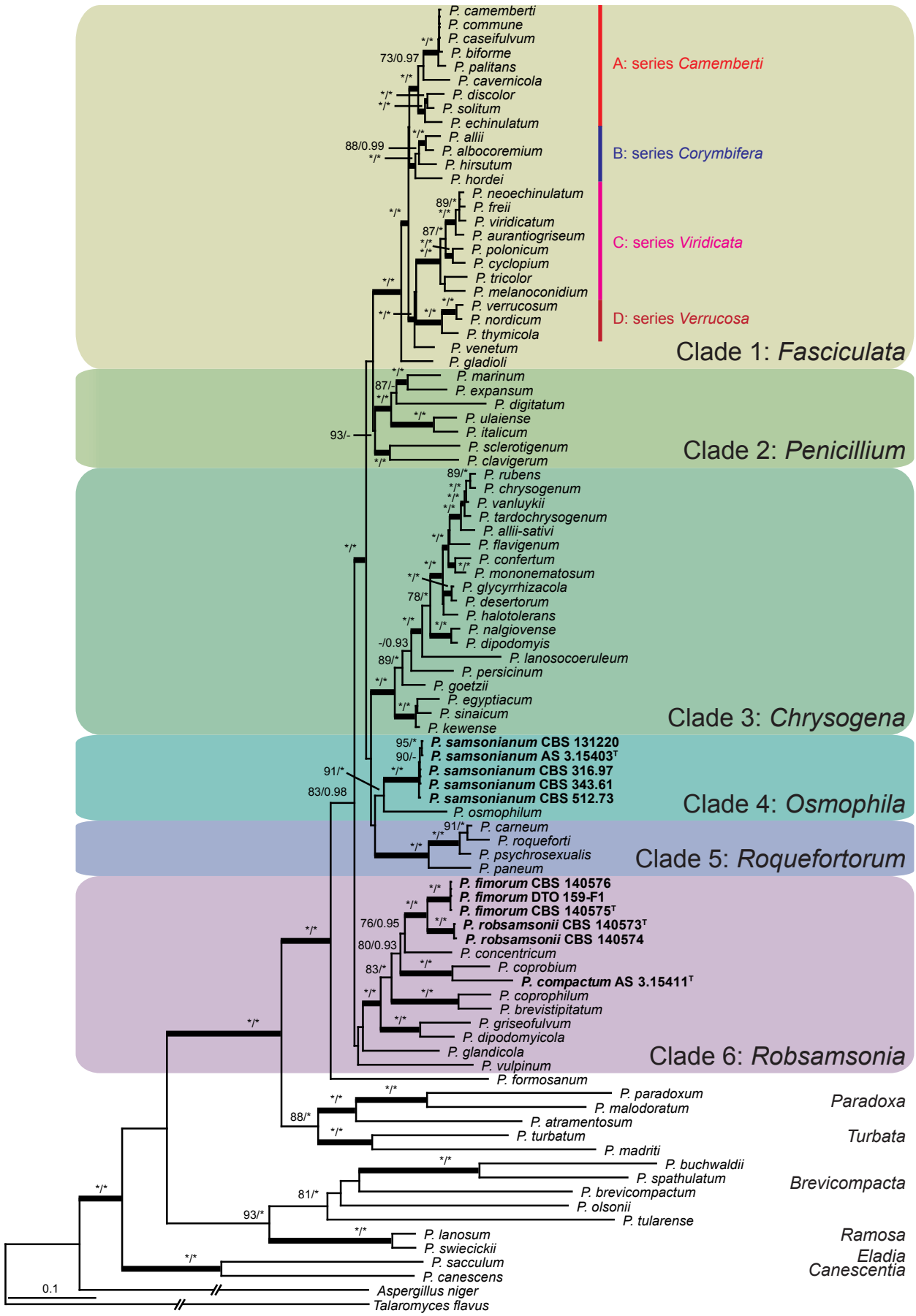

Fig. 1 Best-scoring Maximum Likelihood tree using RAxML based on a combination of partial *BenA*, *CaM* and *RPB2* sequences, showing the relationship among members of *Penicillium* sections *Fasciculata*, *Penicillium*, *Digitata*, *Chrysogena*, *Roquefortorum*, *Turbata*, *Brevicompacta*, *Paradoxa*, *Ramosa*. The bootstrap values of the ML analysis and the BI posterior probabilities values are presented at the nodes (bs/pp). Values less than 70 % supported in the ML analysis or less than 0.95 in the BI analysis are omitted, whereas asterisks (*) indicate full support (100 % bs; 1.00 pp). The branches with more than 95 % bootstrap support and 1.00 pp values are thickened. The phylogram is rooted with *Talaromyces flavus* (CBS 310.38^{T}).

alignment was 1 850 characters (*BenA*, 450 bp; *CaM*, 597 bp; *RPB2*, 803 bp). Before combining the datasets, the most suitable model was calculated and the general time reversible (GTR) plus gamma (+G) was most suitable for each individual dataset. The phylogenies based on the ML and BI analysis were identical, and differences were only in the degree of support. The result of the ML analysis is shown in Fig. 1.

Representatives of each section of subgenus *Penicillium* were included in the analysis and a large species sampling was included for sections *Chrysogena*, *Digitata*, *Fasciculata*, *Penicillium* and *Roquefortorum*. Based on their four-gene phylogeny, Houbraken & Samson (2011) suggested that these five sections are phylogenetically related and this was confirmed in our present analysis. Our results are incongruent with those of Samson et al. (2004). They used partial *BenA* sequences to support an infra-subgeneric classification into sections and series and concluded that because their phylogram covering subgenus *Penicillium* lacked bootstrap support at important nodes, analysis of additional genes should be explored. These differences might also be a consequence of e.g. alignment problems of the *BenA* dataset, the parsimony-based phylogenetic analysis employed, taxon sampling and/or choice of outgroup.

In our analysis, the node grouping strains belonging to sections *Chrysogena*, *Digitata*, *Fasciculata*, *Penicillium* and *Roquefortorum* as defined by Houbraken & Samson (2011) was well supported (Fig. 1) (83 % ML, 0.98 pp). *Penicillium formosanum* and sections *Paradoxa* and *Turbata* occupied a basal position to this large group of strains. Six well-supported clades (> 90 % bs, 1.00 pp) are present in this major lineage. Clade 2 was well supported in the ML analysis (93 %), but poorly in the Bayesian analysis (0.89 pp; data not shown).

Extrolites

Penicillium species belonging to clades 1–6 (Fig. 1) produce various bioactive extrolites, some distributed over many species. Roquefortine C is the most common and is found in all clades (Frisvad et al. 2004). Species in sections basal to clades 1–6 (*Penicillium formosanum*, sections *Brevicompacta*, *Ramosa* and *Turbata*) do not produce roquefortine C or the biosynthetically related extrolites meleagrin, oxaline or neoxaline, while species in section *Paradoxa* sometimes do (*P. malodoratum* and *P. crystallinum*) (Frisvad et al. 2004).

Certain compounds are specific for one of the six sections (Table 2), although individual extrolites may appear in other *Penicillium* species and even in *Aspergillus*. However, no single extrolite occurs in all species of any section. For example, the anticholerolemic agents pyripyropens are only produced by certain clade 6 species (*P. coprobium*, *P. coprophilum*, *P. concentricum* and two new species, *P. compactum*, *P. robsamsonii*), and patulodin and cyclopiamin have only been found in *P. griseofulvum*, *P. concentricum* and *P. glandicola* (clade 6). Terrestric acid, anacins, verrucofortine, pseurotins, viridic acid and auranthine are examples of extrolites that are only present in section *Fasciculata*, but not any of the other five sections treated here.

Patulin-production is common in clades 2, 4–6, rare in clade 1 (one out of 29 species; *P. gladioli*) and absent in *Chrysogena*. The ability of *P. formosanum* to produce patulin is interesting as this species is basal to clades 1–6. However, patulin-producers are on the other hand absent in the basal sections *Turbata*, *Brevicompacta* and *Paradoxa* (Frisvad et al. 2004, Houbraken & Samson 2011, Frisvad et al. 2013). This suggests that patulin production might have been once a trait of all the species, and lost in the non-producers. Genome sequencing of these species might indicate whether the gene cluster for patulin has been lost, silent, or whether it never has been present in those species, or even acquired by horizontal gene transfer in the patulin producing species. Alternatively these species could be cultivated on PDA with manganese, an optimal medium for patulin production (Dombrink-Kurtzman & Blackburn 2005), as it is still possible they may produce patulin under optimal conditions.

Morphology, physiology and ecology

The majority of species belonging to clades 1–6 (Fig. 1) predominantly have ter- and/or quarterverticillate conidiophores, and can be differentiated by phenotypic and/or physiological characters (Frisvad & Samson 2004). Important macroscopic features for identification are e.g. growth rates on agar media (CYA, YES, MEA, CYAS, CREA), obverse and reverse colony colours, colony texture and colony diameters after incubation at various temperatures (e.g. 15, 30, 37 °C). Stipe ornamentation and shape, size and ornamentation of conidia are frequently used characters to distinguish these species microscopically. In addition to the use of these characters in species delimitation, they are useful to classify species in series, but are more difficult to apply to a sectional classification. The sectional classification of Houbraken & Samson (2011) was based on a multigene phylogeny, and a limited number of characters were subsequently linked to each section. Here we list combinations of characters that can be used to characterize the sections investigated in this study (Table 2).

Frisvad & Samson (2004) reported on the association between *Penicillium* species and their natural habitat. Some of the investigated species here have a strong association with a specific substrate. For example, *P. italicum*, *P. ulaiense* and *P. digitatum* are strongly associated with citrus fruits, *P. tulipae* with tulip bulbs and *P. allii* with garlic. In contrast, other species occur in a wider habitat range, e.g. *P. expansum* is known as a causal agent of rot of pomaceous fruits, but can also be isolated from different habitats (e.g. dried meat, nuts). The ecology of the investigated group of Penicillia and the phylogeny presented in Fig. 1 generally correlate well. An overview of the investigated sections and details on the ecology of the species belonging to these sections is given in Table 2.

Sectional classification: phylogeny, morphology and ecology

Section Fasciculata (clade 1)

Clade 1 mainly contains species previously assigned to section *Fasciculata*, the only exception being *P. gladioli*, a species previously classified in section *Penicillium*, series *Gladioli* (Frisvad & Samson 2004, Houbraken & Samson 2011) (Fig. 1). Most species in section *Fasciculata* have rough-walled conidiophore stipes and (sub)globose conidia. These conidia can be smooth to distinctly roughened, and the latter feature is only observed in this clade. Most species of this section grow well at 15 °C, 25 °C (except those in series *Verrucosa*), and at low water activities. The classification of *P. gladioli* in *Fasciculata* is confirmed by phenotypic characters, because it grows well at 15 °C, and produces rough-walled stipes and subglobose conidia. On the other hand, this species is unique as it is the only member of this section that is able to produce patulin.

Section *Fasciculata* contains species that commonly occur on stored or manufactured foods. However, a further subdivision (series classification) can be made here. Species belonging to series *Camemberti* (Fig. 1, clade A) typically occur on proteinaceous and lipid-containing foods. Clade B (series *Corymbifera*) contains species mainly associated with flower bulbs and occasionally other plant roots. The species belonging to clade C (series *Viridicata*) are typically associated with stored cereal grains and those belonging to series *Verrucosa* (clade D) are associated with stored cereal grains (*P. verrucosum*) and dried or salted meat products (*P. nordicum*) (Frisvad & Samson 2004).

Table 2 Phenotypic, physiology, ecology and extrolite data linked to the sections proposed in this study.

Clade	Section	Phenotype and physiology	Ecology	Section specific extrolites*
1	*Fasciculata*	Good growth on CYA15 °C (psychrotolerant), 25 °C (except those in series *Verrucosa*), and at low water activities. Conidiophores rough-walled; conidia smooth- or rough-walled, (sub)globose.	Common on stored or manufactured foods (e.g. stored cereals, cheese, nuts, and other fat and protein rich substrates). Also occurring on flower bulbs, root vegetables and onions.	Aurantiamin, anacins, verrucins, terrestric acids, ochratoxins, glyanthrypine, verruculones, verrucofortins, daldinins, atrovenetins, auranthine, pseurotins, rugulovasines, territrems, puberulonic acid, compactins, lumpidin, viridic acid, alantrypinone are only found in this section. Penicillic acid (exception: one strain of *P. carneum*, sect. *Roquefortorum*), compactins (exception: *P. lanosum*, sect. *Ramosa*), xanthomegnins (exception: *P. clavigerum*, sect. *Robsamsonia*), viridicatols (exception: *P. vulpinum*, sect. *Robsamsonia*). Chaetoglobosins are shared with *Penicillium* and *Robsamsonia*, fulvic acid is shared with *Robsamsonia* and *Penicillium*, chrysogine is shared with *Chrysogena* and *Robsamsonia*, sclerotigenin is shared with *Penicillium*, brevianamides are shared with *Brevicompacta*, cyclopiazonic acid is shared with *Robsamsonia* and *P. clavigerum*, cyclopaldic acid is shared with *Roquefortorum* and *Chrysogena*, penitrems are shared with *Penicillium*, *Chrysogena*, *Robsamsonia*, asteltoxin shared with *Formosana*, *Chrysogena* and *Robsamsonia*, dipodazin shared with *Chrysogena*, palitantin shared with *Robsamsonia*.
2	*Penicillium*	Colony texture often strongly fasciculate or synnematous. Conidiophores smooth-walled; conidia smooth-walled, ellipsoidal (occasionally subglobose).	Plant pathogenic species: rot in pomaceous and citrus fruits, yams.	Tryptoquialanines, gladiolic acid, italinic acid, pentostatins, communesins, expansolide are only found in this section. Griseofulvin is shared with *Robsamsonia* and *Chrysogena*, verrucolone shared with *Fasciculata* and *Brevicompacta*.
3	*Chrysogena*	Colony texture velutinous to weakly floccose; good growth on CYA30°C, CYA : CYAS > 1. Conidophores bi-, ter- or quarter-verticillate, divergently branched; stipes smooth-walled; phialides relatively short (< 9 µm); conidia smooth or at most finely roughened.	Dry habitats, e.g. desert and Artic soil; indoor environments. Salt tolerant.	Sorbicillins, xanthocillins, secalonic acids, fumitremorgins, isochromantoxin, nalgiovensin, viridicatumtoxin are only found in this section. Penicillin is shared with *Robsamsonia*, PR-toxin shared with *Roquefortorum*.
4	*Osmophila*	Good growth on CYA15°C (and poor or absent on CYA30°C); CYA:CYAS ratio around 1. Conidiophores smooth-walled.	Soil.	No section specific extrolites known. Andrastin A is shared with *Fasciculata*, *Penicillium*, *Roquefortorum*, and *Robsamsonia*.
5	*Roquefortorum*	Velutinous colonies; spreading on CYA and MEA; growth on MEA supplemented with 0.5 % acetic acid. Conidiophores coarsely roughened.	Symbiotic relationship with lactic acid bacteria and certain acid-tolerant yeasts (Samson et al. 2002).	Marcfortins, botryodiploidin, isofumigaclavine are only found in this section. Penitrems are shared with *Fasciculata*, *Penicillium* and *P. glandicola*, mycophenolic acid is shared with *Osmophila* and *Brevicompacta*, patulin is shared with *Robsamsonia*, *Penicillium*, *Osmophila*, and *Fasciculata*, PR-toxin and eremofortins shared with *Chrysogena*.
6	*Robsamsonia*	Moderately fast growth on CYA incubated at 25 °C (15–32 mm); slow or absence of growth on CYA30°C. Conidia smooth-walled, (broadly) ellipsoidal.	Mainly dung, also on dry cereals and seeds (*P. griseofulvum*, *P. dipodomyicola*).	Pyripyropens, patulodin, alternariol, fulvic acid, mycelianamide and cyclopiamine appear to be unique for this section. Barceloneic acid is shared with *Fasciculata*, quinolactacin is shared with *Brevicompacta*.

* Comparison between the species belonging to one of the six sections listed in this Table. Some of the section specific compounds can be produced by species outside these sections.

Section Penicillium (clade 2)

Penicillium expansum, the type species of section *Penicillium*, belongs to clade 2 (Fig. 1). Other species previously assigned to section *Penicillium* and confirmed as belonging to this clade are *P. marinum*, *P. ulaiense*, *P. italicum*, *P. sclerotigenum* and *P. clavigerum*. *Penicillium coccotrypicola* is placed, based on the *BenA* sequence deposited in GenBank (KM605437), in section *Penicillium*. Several species previously classified in section *Penicillium* do not belong to clade 2 and are mainly distributed in clade 6 (Frisvad & Samson 2004, Houbraken & Samson 2011). Further, phylogenetic analysis shows that *P. digitatum*, the type species of section *Digitata*, belongs to clade 2.

Section *Penicillium* is phenotypically diverse and there are only a few uniting characters, such as the production of smooth-walled stipes, and smooth-walled, ellipsoidal (or subglobose) conidia. Several species have a strongly fasciculate or synnematous colony texture (*P. clavigerum*, *P. coccotrypicola*, *P. expansum*, *P. italicum*, *P. ulaiense*). The synnematal structure of *P. clavigerum* was discussed by Frisvad & Samson (2004) who noted differences between this and other *Penicillium* species. A number of subgenus *Penicillium* species (e.g. *P. coprophilum*, *P. glandicola*, *P. vulpinum*) produce determinate synnemata (synnemata consisting of a more or less sterile stalk with a fertile capitulum), while *P. clavigerum* (and *P. coccotrypicola*) form indeterminate synnemata (synnemata covered over nearly the entire length with conidiophores). Based on our phylogenetic data it can be speculated that the characteristic synnemata formation in *P. clavigerum* is evolutionary related with the fascicule (coremiforme) structures present in species in clade 2.

The species classified by Frisvad & Samson (2004) in section *Penicillium* are associated with various substrates (dung, dry cereals, fruits). Our results show that this section mainly contains plant pathogenic species. For example, *P. sclerotigenum* causes rot in yam tubers, and *P. digitatum*, *P. italicum* and *P. ulaiense* rot of citrus fruits. *Penicillium expansum* is associated with rot in pomaceous fruits; however, it also occurs on other substrates, such as nuts, oilseeds, soil and wood. Based on these ecological data, it is expected that all species of this section will be good pectinase producers.

Table 3 Overview of extrolites produced by species belonging to *Penicillium* section *Robsamsonia**.

Species	patulin	griseofulvin	pyripyropens	patulodin	meleagrin	roquefortine C	cyclopiamin	quinolactacin	Cyclopiazonic acid
P. brevistipitatum	–	–	–	–	–	–	–	–	–
P. compactum	+	–	+	–	+	+	–	+	–
P. concentricum	+	–	+	+	+	+	+	–	–
P. coprobium	+	–	+	–	+	+	+	–	–
P. coprophilum	–	+	+	–	+	+	–	–	–
P. dipodomyicola	+	+	–	–	–	–	–	–	+
P. fimorum	–	–	–	–	–	–	–	–	–
P. glandicola	+	–	–	+	+	+	–	–	–
P. griseofulvum	+	+	–	+	–	+	+	–	+
P. robsamsonii	–	–	+	–	–	+	–	+	–
P. vulpinum	+	–	–	–	+	+	+	–	–

* Some extrolites are only produced by one species in this section: andrastin A, citreoisocoumarin, palitantin, and xanthoepocin in *P. fimorum*; clavatols and chaetoglobosins in *P. robsamsonii*, barceloneic acid and asteltoxin in *P. concentricum*, alternariol in *P. coprophilum*, fulvic acid and mycelianamide in *P. griseofulvum*, penitrem A in *P. glandicola* and pachybasin, lichexanthone and viridicatins in *P. vulpinum*. Only new unique extrolites were detected in *P. brevistipitatum* (see data generated here in conjunction with Frisvad & Samson 2004).

The extrolites tryptoquialanines, gladiolic acid, italinic acid, pentostatins, communesins, expansolide are only found in section *Penicillium* and not in the other five sections studied here (Table 3). *Penicillium expansum*, *P. marinum*, *P. sclerotigenum* and *P. clavigerum* are able to synthesize patulin. The former two species are phenotypically and phylogenetically related, as are *P. sclerotigenum* and *P. clavigerum*. No patulin production was observed in the other three species of this section (*P. digitatum*, *P. italicum*, *P. ulaiense*).

Section Chrysogena (clade 3)

The phylogenetic relationship of species belonging to section *Chrysogena* was investigated in detail by Houbraken et al. (2012a) and those results are confirmed in this study. Species belonging to this section generally produce velutinous to weakly floccose colonies, grow rather fast on CYA incubated at 25 and 30 °C, and have a CYA:CYAS ratio above 1. Microscopically, they produce bi-, ter- or quarterverticillate, divergently branched, smooth-walled conidiophores, relatively short phialides (< 8 µm), and smooth or at most finely roughened conidia.

Various species of section *Chrysogena* commonly occur in indoor environments, but they are also isolated from dry habitats such as desert or Arctic soil (Houbraken et al. 2012a). This suggests that this group of species thrives well in extreme environments.

Section Osmophila (clade 4)

Clade 4 includes *P. osmophilum* and a putative new species named here *P. samsonianum* (see Taxonomy section) (Fig. 1). These two species share several similarities. For example, both species grow moderately fast on CYA and YES (14–26 mm and 14–32 mm, respectively), growth is equally fast on CYA, CYAS and CYA15°C, while growth on CYA30°C is slow. Furthermore, they produce bi-, ter- and quarterverticillate branched, smooth-walled conidiophores, and smooth-walled conidia. *Penicillium osmophilum* and *P. samsonianum* were isolated from soil and their primary habitat is unknown. No section specific extrolites are found and e.g. andrastin A is shared with *Fasciculata*, *Penicillium*, *Roquefortorum* and *Robsamsonia*. Patulin production was detected in *P. samsonianum*, but not in the sister species *P. osmophilum*.

Section Roquefortorum (clade 5)

Species previously assigned to section *Roquefortorum* belong to clade 5 (Houbraken & Samson 2011). This section includes species that are spreading on CYA and MEA, and are able to grow on MEA supplemented with acetic acid. The conidiophores of these species are often coarsely ornamented, occasionally smooth, and they produce large conidia measuring 3.5–5 µm diam.

Section *Roquefortorum* species grow well at low oxygen and high carbon dioxide levels, low pH, and in the presence of organic acids. These species are therefore also predominating on lactic acid fermented substrates (e.g., silage, cheese, salami) and acid environments (e.g., rye bread).

Section Robsamsonia (clade 6)

Clade 6 contains species previously assigned to series *Urticicolae* (*P. dipodomyicola* and *P. griseofulvum*) and *Claviformia* (*P. brevistipitatum*, *P. clavigerum*, *P. concentricum*, *P. coprobium*). *Penicillium glandicola* and *P. vulpinum*, classified in series *Claviformia* (Frisvad & Samson 2004) were basal to clade 6 but lacked support in the ML and Bayesian analysis (Fig. 1). The species in this section share phenotypic similarities. Growth of these species is moderately fast on CYA incubated at 25 °C (15–32 mm) and slow or absent on CYA at 30 °C. Microscopically, most of the members share the production of smooth-walled, (broadly) ellipsoidal conidia. Furthermore, various members of this section (*P. coprophilum*, *P. glandicola*, *P. vulpinum*) produce synnematous structures on their (natural) substrate.

Clade 6 mainly contains species that are associated with dung (*P. coprophilum*, *P. coprobium*, *P. concentricum*) or dry cereals and seeds (*P. griseofulvum*, *P. dipodomyicola*). *Penicillium glandicola* and *P. vulpinum* are also isolated from dung or dungy soil. Based on the occurrence of these species on a similar habitat (dung) as other members of this section and the (weak) phylogenetic support, we decided to accommodate these two species in section *Robsamsonia*.

New sectional classification

Based on the presented phylogeny, the extrolite data, phenotypic observations, physiology and ecology (Fig. 1, Table 2), we treat the six phylogenetic clades as separate sections: *Fasciculata*, *Penicillium*, *Chrysogena*, *Roquefortorum*, *Osmophila* (clade 4) and *Robsamsonia* (clade 6). The latter two sections are formally introduced in the Taxonomy section of this manuscript. Section *Digitata* is placed in synonymy with section *Penicillium*. The type strain of *Penicillium formosanum* (IBT 19748 = IBT 21527 = CBS 211.92 = CBS 101028) forms a separate lineage. This species produces yellow synnemata on MEA and oatmeal agar (Frisvad & Samson 2004), a feature not observed in any other species belonging to clade 1–6. This species might represent a separate section, but description is deferred until new species related to *P. formosanum* are found

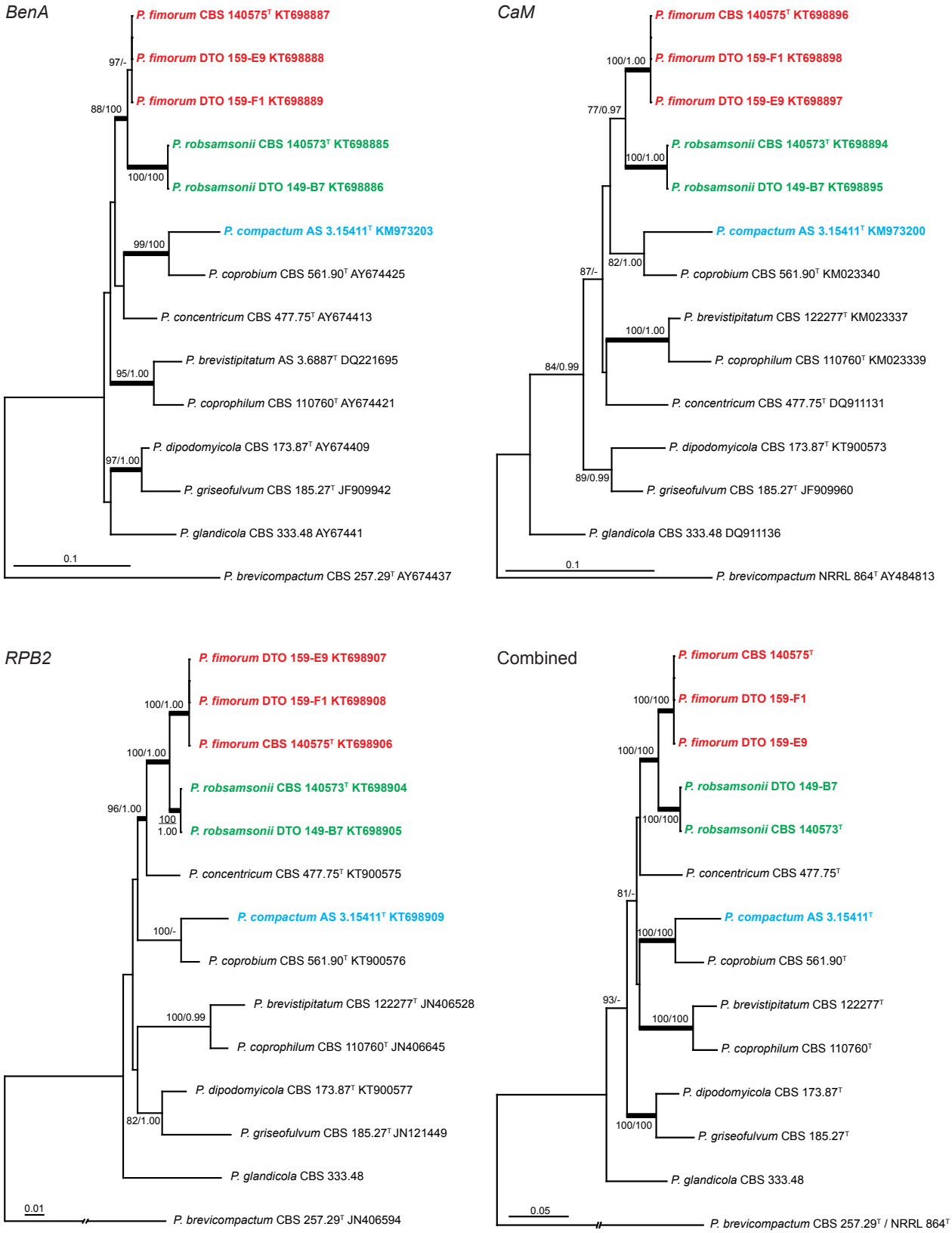

Fig. 2 Best-scoring Maximum Likelihood tree based *BenA*, *CaM* and *RPB2* datasets sequences using RAxML. Well-supported branches (> 95 % bootstrap support and 1.00 posterior probability) are in **bold**; values less than 70 % bs and lower than 0.95 pp are not shown and an asterisk (*) indicate full support (100 % bs; 1.00 pp). The bootstrap percentages and the BI posterior probabilities values are presented at the nodes (bs/pp). The phylogram is rooted with the type strain of *Penicillium brevicompactum* (CBS 257.29^{T} or NRRL 864^{T}).

Table 4 Overview of phenotypic characters of species belonging to section *Robsamsonia*.

Species	Colony diam. CYA (mm)	Colony diam. YES (mm)	Reverse colour CYA	Reverse colour YES	Conidium colour on MEA	Growth on CREA	Ehrlich reaction	Stipe ornamentation	Length phialides (µm)	Size conidia (µm)
P. brevistipitatum	34–38	43–47	Brown	Light brown with brown centre	Dull green	Good	–	Smooth	8–11	2.7–3.5 × 3.5–4.5
P. compactum	17–23	29–35	Dark blackish brown	Dark brown, brown	Dark dull green	Good	–	Smooth	9–13	3.5–4 × 4–4.5
P. concentricum	15–24	25–32	Orange to orange-red	Yellow to strongly orange	Blue-green	Good	–	Smooth	8–11	2.5–3.3 × 3–4.5
P. coprobium	20–26	29–39	Greyish brown to yellow-brown	Cream colored to curry to olive	Dark dull green	Good	–	Smooth	8–10	2.5–3.5 × 3–4
P. coprophilum	23–30	34–47	Dark brown	Curry to brown-yellow	Greyish green	Good	–	Smooth	7–10	2.5–3.3 × 2.5–4
P. dipodomyicola	23–30	35–43	Brown or dark brown	Brown to dark brown	Dull green	Weak	+	Smooth	5–7	2.7–3.5 × 2.5–3.5
P. fimorum	20–26	30–37	Brown	Brown	Dull to dark green	Weak	–	Rough	8–10	3.7–4.5 × 3–4
P. glandicola	17–30(–35)	19–36	Orange-brown or brown	Bright orange-red	Grey-green or pure green	Good	–	Rough	7.5–10.5	2.5–3.5 × 2–3
P. griseofulvum	24–32	32–42	Crème to crème brown, beige brown	Yellow-brown or brown	Grey-green	Weak	+	Smooth	5–7	2.3–3 × 2.7–4
P. robsamsonii	17–22	31–39	Pale brown	Pale brown	Dull green	Moderate	+	Rough	7.0–8.5(–9.5)	2.5–3.5 × (3.0–)3.5–4.3
P. vulpinum	17–28	25–35(–40)	Light yellow, beige, brown or reddish brown	Yellow-brown or pale brown	Grey-green or dull green	Good	– (+)	Smooth	9–13	2.5–4 × 3–4.5

and studied. With the introduction of two new sections and the synonymizing of section *Digitata*, there are currently 26 accepted sections in *Penicillium* (Houbraken & Samson 2011).

New species in section Robsamsonia

Phylogeny

The phylogeny of the new species belonging to section *Robsamsonia* was studied in detail by comparing single gene and combined phylogenies based on partial *BenA*, *CaM* and *RPB2* sequences. The analysis included 14 isolates, including the outgroup species. The concatenated alignment was 1 638 bp long (*BenA*: 353 bp; *CaM:* 482 bp; *RPB2*: 803 bp). The Kimura 2-parameter with gamma distributed sites (+G) was the most optimal model for the *BenA* dataset, and the general time Reversible (GTR+G) model was most optimal for the *CaM* and *RPB2* datasets. No significant differences were observed between the ML and BI analyses. Nine species can be confidentially placed in this section, including the three new species (*P. fimorum*, *P. robsamsonii* and *P. compactum*) described in this manuscript. Good statistical support in the phylogram is often present at species level, and poor in the deeper nodes. *Penicillium fimorum* and *P. robsamsonii* are phylogenetically related, and *P. compactum* is related to *P. coprobium*. *Penicillium brevistipitatum* and *P. coprophilum* form a lineage, as do *P. dipodomyicola* and *P. griseofulvum*. The phylogenetic relationship of *P. concentricum* with other members of this section is unclear. This species is basal to *P. robsamsonii* and *P. fimorum* in Fig. 1 (weak statistical support; 76 % bs, 0.95 pp), but this relationship was not found in the individual and combined analyses of section *Robsamsonia* species only (Fig. 2).

Morphology

The species belonging to section *Robsamsonia* share phenotypic similarities, such as a moderately fast growth rate on CYA incubated at 25 °C (15–32 mm) and slow or absence of growth on CYA30°C. Microscopically, most of the members share the production of smooth-walled, (broadly) ellipsoidal conidia. Various characters can be used to distinguish the species belonging to this clade. *Penicillium griseofulvum* and *P. dipodomyicola* are phenotypically deviating from the other species in this section and the most typical features of these species are the production of divergently branched conidiophores with short phialides (< 7 µm). The production of rough walled conidiophore stipes can be used to distinguish *P. fimorum* and *P. robsamsonii* from the other species. Differences are also observed in conidium colour on MEA, and reverse colour on CYA and YES. For example, *P. compactum* produces a dark brown reverse on CYA and YES and dark dull green conidia on MEA. An overview of diagnostic features is given in Table 3. These characters appear to be stable and can be used to distinguish each species morphologically. Details are also given in the notes listed with the descriptions of the new species in the Taxonomy section.

Extrolites

The species of section *Robsamsonia* share various extrolites, but can also be differentiated by their different extrolite profiles. For example, *P. compactum* and *P. coprobium* share pyripyropens, patulin and meleagrin, but differ in that *P. compactum* produces quinolactacin and *P. coprobium* produces cyclopiamin (Frisvad et al. 2004). *Penicillium robsamsonii* and *P. fimorum*, both from mouse pellets, share production of andrastin A. On the other hand, *P. robsamsonii* produces the extrolites chaetoglobosins, pyripyropens, patulodin and quinolactacin (the latter which is shared with *P. compactum*), while *P. fimorum* is different in that it produces citreoisocoumarin, palitantin and xanthoepocin. Both species produce as yet unknown extrolites, which may be new drug-lead candidates as these copro-

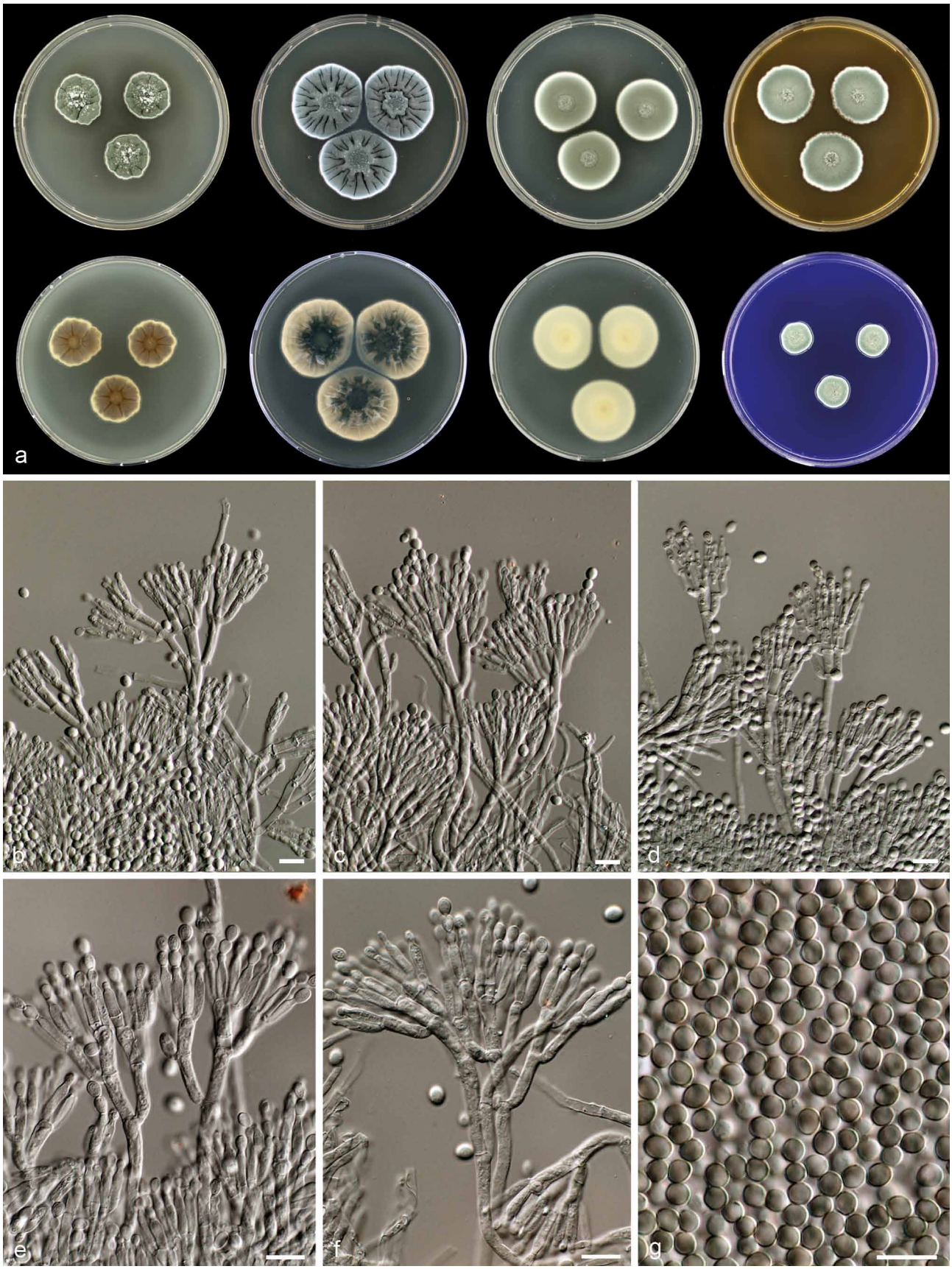

Fig. 3 *Penicillium compactum*, CBS 138918^{T} a. 7-d-old cultures at 25 °C, left to right, first row, all obverse, CYA, YES, DG18, MEA; second row, CYA reverse, YES reverse, DG18 reverse, CREA obverse; b–f. conidiophores; g. conidia. — Scale bars = 10 µm.

philic species often produce a series of bioactive compounds (Bills et al. 2013). Section *Robsamsonia* is diverse regarding patulin production and seven of the 11 species are producers: *P. concentricum*, *P. coprobium*, *P. compactum*, *P. griseofulvum* and *P. dipodomyicola*, *P. glandicola* and *P. vulpinum*. The latter two species potentially belong to this section (see Taxonomy section). It should be further investigated whether the non-producers can produce patulin on PDA with manganese, an optimal medium for patulin production (Dombrink-Kurtzman & Blackburn 2005). An overview of extrolites that are produced by species in this section is given in Table 4.

TAXONOMY

Our analysis revealed the presence of two new sections and four new species in subgenus *Penicillium*. These sections and species are described below.

Section ***Osmophila*** Houbraken & Frisvad, *sect. nov.* — MycoBank MB 815869

In: subgenus *Penicillium*.

Type. *Penicillium osmophilum* Stolk & Veenb.-Rijks, Antonie van Leeuwenhoek 40: 1. 1974. MB319288.

Etymology. Referring to *Penicillium osmophilum*, the type species of the section.

Diagnosis — This section is phylogenetically distinct (Fig. 1). Species in this section produce bi-, ter and quarterverticillate conidiophores and have similar growth rates on CYA incubated at 15 and 25 °C. Furthermore, growth on CYA incubated at 30 °C is restricted and the CYAS:CYA ratio is around 1.

Notes — The following species are included in this section: *Penicillium osmophilum* and *P. samsonianum*.

Section ***Robsamsonia*** Houbraken & Frisvad, *sect. nov.* — MycoBank MB815870

In: subgenus *Penicillium*.

Type. *Penicillium robsamsonii* Frisvad & Houbraken, this study. MB815872.

Etymology. Referring to a noun dedicated to Rob Samson, also used in *Penicillium robsamsonii*, the type species of this section.

Diagnosis — This section is phylogenetically distinct (Fig. 1). The majority of the species in this section are coprophilic and most members form smooth-walled, ellipsoidal conidia and produce patulin, pyripyropens, patulodin and/or cyclopiamin.

Notes — *Penicillium glandicola* and *P. vulpinum* are placed without statistical support in a basal position to the species belonging to clade 6 (Fig. 1). They are tentatively included in section *Robsamsonia*. The following 11 species are included in this section: *Penicillium brevistipitatum*, *P. compactum*, *P. concentricum*, *P. coprobium*, *P. coprophilum*, *P. dipodomyicola*, *P. fimorum*, *P. glandicola*, *P. griseofulvum*, *P. robsamsonii* and *P. vulpinum*.

Penicillium compactum L. Wang & Houbraken, *sp. nov.* — MycoBank MB810216; Fig. 3

In: *Penicillium* subgenus *Penicillium* section *Robsamsonia.*

ITS barcode. KM973207 (alternative markers: *BenA* = KM973203; *CaM* = KM973200; *RPB2* = KT698909).

Etymology. The species is named in relation to its compact conidiophores.

Type specimen. China, Heilongjiang, Tangyuan County, Daliangzihe forest farm, N46°49'21" E129°58'04", 312 m; ex soil sample under *Pinus koraiensis*, no. HLJ96, 29 Aug 2014, *L. Wang* (holotype HMAS 245701, cultures ex-type AS3.15411 = CBS 138918 = IBT 33393 = DTO 316-B8).

Diagnosis — *Penicillium compactum* is characterised by its appressed, terverticillate conidiophores, large (4–4.5 × 3.5–4.0 µm), broadly ellipsoidal conidia and dark brown reverse on YES.

Description — Colony diam, 7 d, in mm: CYA 17–23; CYA15°C 12–18; CYA30°C 5–12; CYA37°C no growth; MEA 22–28; YES 29–35; CYAS 29–35; creatine agar 10–17, good growth, acid production absent.
CYA, 25 °C: Colonies elevated in centre; sporulation strong; colony texture granular; mycelium white; exudate absent; soluble pigments present, light brown; radial sulcate; margin irregular; conidia dark dull green; reverse dark blackish brown. YES, 25 °C: Sporulation strong; mycelium white; exudate absent; soluble pigments absent; conidia dull green; reverse dark brown in centre; edges brown. MEA, 25 °C: Sporulation strong; colony texture velvety, slightly floccose in centre; mycelium white; exudate present, small, clear; soluble pigments absent; conidia dark dull green; reverse brown. DG18, 25 °C: Sporulation strong; colony texture velvety; mycelium white; conidia dull green; reverse pale yellow in centre; edge transparent. Ehrlich reaction negative.
Sclerotia absent. *Synnemata* absent. *Conidiophores* arising from substrate, (40–)50–80(–100) µm long, smooth-walled, terverticillate, 4.5–6(–7) µm wide. *Rami* 1–4 per stipe, appressed, 10–15(–18) × 4.5–6 µm. *Metulae* 2–4(–6) per ramus, 9–14(–18) × 3–4 µm. *Phialides* (2–)4–6 per metula, cylindrical with short collula, 9–13 × 2–3 µm. *Conidia* born in short loosely tangled chains, smooth-walled, ellipsoidal, 4–4.5 × 3.5–4.0 µm.
Extrolites — Meleagrin, patulin, quinolactacin and three different pyripyropens.

Additional material examined. China, Heilongjiang, Harbin, Xiaojia of Songbei District, N46°04'36" E126°14'58", 130 m, ex soil from *Raphanus sativus* farm, no. DB12, 17 Aug. 2001, *L. Wang*, culture AS3.6674.

Penicillium fimorum Frisvad & Houbraken, *sp. nov.* — MycoBank MB815871; Fig. 4

In: *Penicillium* subgenus *Penicillium* section *Robsamsonia.*

ITS barcode. KU904342 (alternative markers: *BenA* = KT698889; *CaM* = KT698898; *RPB2* = KT698908).

Etymology. The name refers to the dung habitat of the species.

Type specimen. Denmark, Høve Strand, ex mouse dung, 2009, *J.C. Frisvad* (holotype CBS H-22342, cultures ex-type CBS 140575 = IBT 29495 = DTO 149-B8 = DTO 159-F1).

Diagnosis — Colonies on CYA velvety to slightly fasciculate in centre with brown reverse colour; stipes rough walled; Ehrlich reaction negative, production of andrastin A, citreoisocoumarin, palitantin and xanthoepocin.

Description — Colony diam, 7 d, in mm: CYA 20–26; CYA15°C 15–20; CYA30°C 8–12; CYA37°C no growth; MEA 20–25; YES 30–37; CYAS 18–22; creatine agar 3–10, weak growth, acid production absent.
CYA, 25 °C: Colonies elevated in centre; sporulation strong; colony texture velvety, slightly fasciculate in centre; mycelium white; exudate present as large pale brown droplets; soluble pigments present, poor, pale brown; radial sulcate, deep; margin entire to slightly irregular; conidia grey-green; reverse brown. YES, 25 °C: Sporulation moderate to good, mycelium white; exudate absent; soluble pigments present, brown; conidia dull green, grey-green in centre; reverse brown. MEA, 25 °C: Sporulation strong; colony texture velvety, slightly fasciculate in centre; mycelium white; exudate present, large, brown droplets; soluble pigments absent; conidia dull green to dark green; reverse brown. DG18, 25 °C: Sporulation strong; colony texture

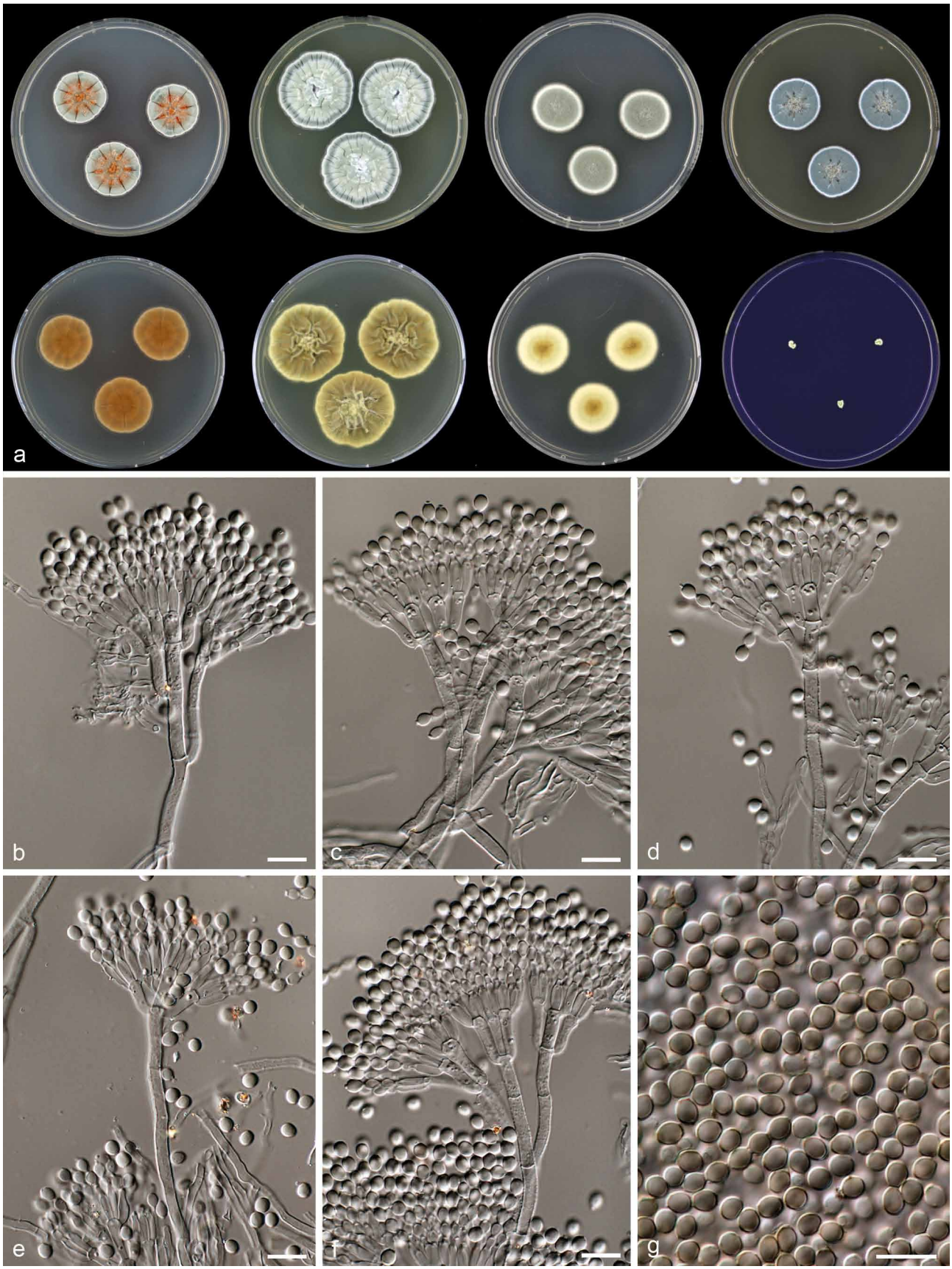

Fig. 4 *Penicillium fimorum*, CBS 140576. a. 7-d-old cultures at 25 °C, left to right, first row, all obverse, CYA, YES, DG18, MEA; second row, CYA reverse, YES reverse, DG18 reverse, CREA obverse; b–f. conidiophores; g. conidia. — Scale bars = 10 µm.

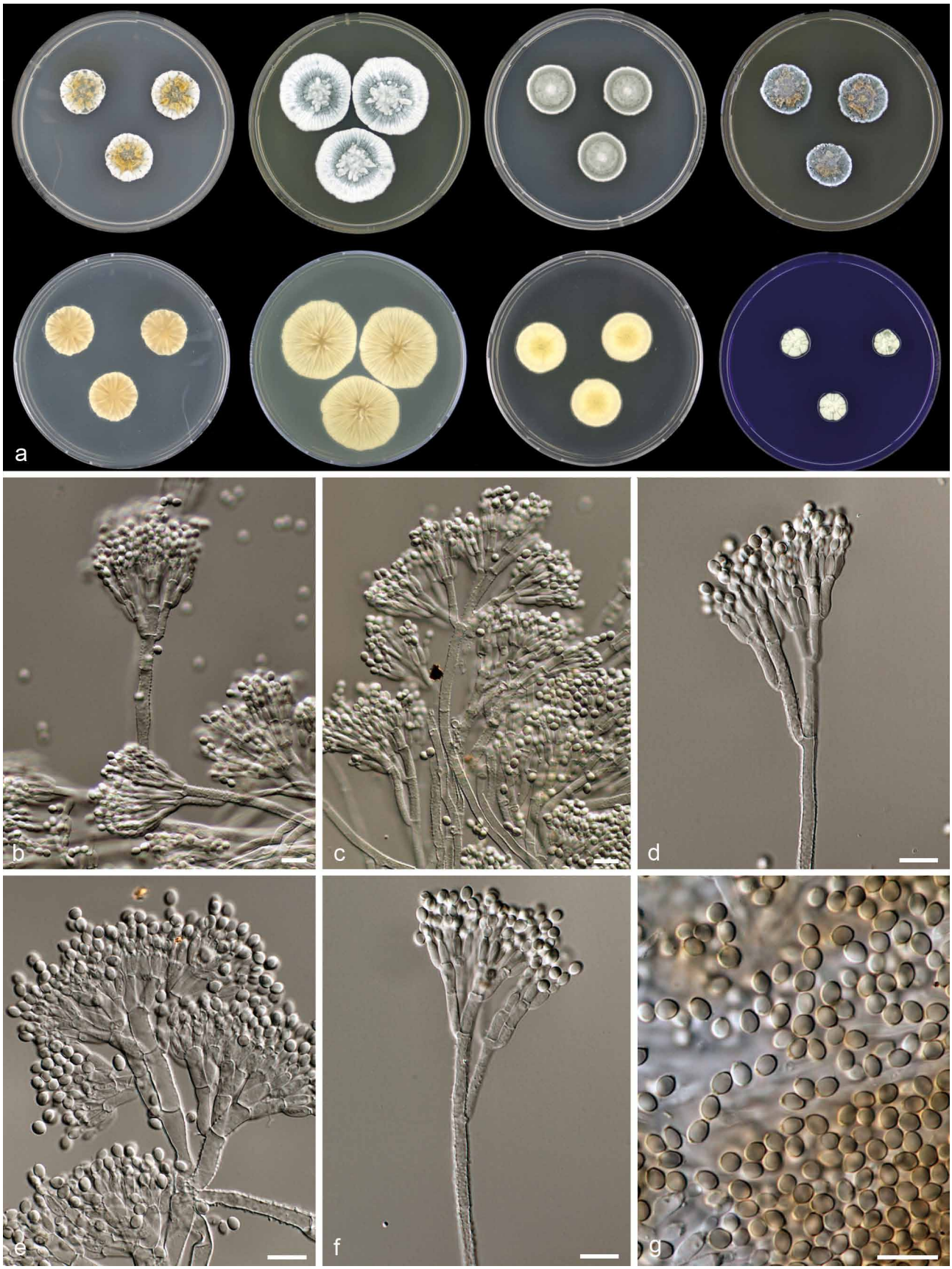

Fig. 5 *Penicillium robsamsonii*, CBS 140573^{T}. a. 7-d-old cultures at 25 °C, left to right, first row, all obverse, CYA, YES, DG18, MEA; second row, CYA reverse, YES reverse, DG18 reverse, CREA obverse; b–f. conidiophores; g. conidia. — Scale bars = 10 µm.

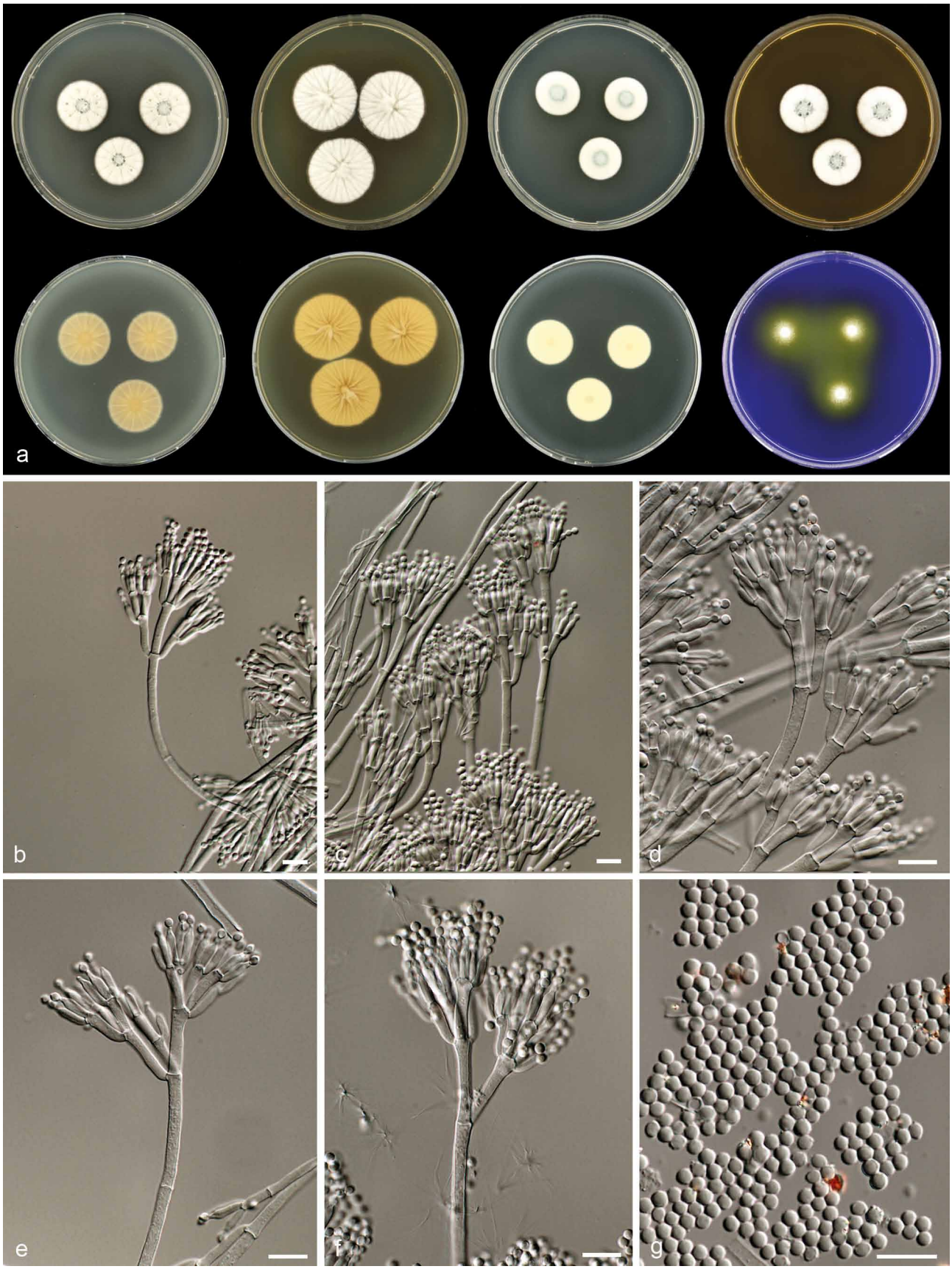

Fig. 6 *Penicillium samsonianum*, CBS 138919[T]. a. 7-d-old cultures at 25 °C, left to right, first row, all obverse, CYA, YES, DG18, MEA; second row, CYA reverse, YES reverse, DG18 reverse, CREA obverse; b–f. conidiophores; g. conidia. — Scale bars = 10 µm.

velvety to slightly floccose; mycelium white, conidia dull green; reverse brown in centre, edge pale brown. Ehrlich reaction negative.
Sclerotia absent. *Synnemata* absent. *Conidiophores* 40–120 µm long, with rough-walled stipes, predominantly terverticillate, occasionally bi- or quarterverticillate, stipe 3.0–4.0 µm wide. *Rami* 1–3 per stipe, slightly appressed, 10–20 × 3.0–4.5 µm. *Metulae* (2–)3–5, 9.5–11.5 × 2.5–4.0 µm. *Phialides* ampulliform, 3–9 per metula, 8–10 × 2.0–3.0 µm. *Conidia* in long, defined chains, smooth-walled, ellipsoid, 3.7–4.5 × 3.0–4.0 µm.

Extrolites — andrastin A, citreoisocoumarin, palitantin, xanthoepocin.

Additional material examined. Denmark, Høve Strand, ex mouse dung, 2009, *J.C. Frisvad*, cultures CBS 140576 = DTO 159-E9 = IBT 31262 and DTO 159-F1 = IBT 29495.

Notes — *Penicillium fimorum* is phylogenetically closely related to *P. robsamsonii*. Colonies of *P. fimorum* are velvety on CYA and have a brown reverse, and *P. robsamsonii* has fasciculate colonies and a pale brown reverse. Furthermore, *P. robsamsonii* has a violet reaction with Ehrlich reagent (due to the production of chaetoglobosins), while the examined *P. fimorum* cultures are negative. Both species also differ in their extrolite profiles (also see Table 4). It is interesting to note that *P. robsamsonii*, *P. glandicola*, *P. vulpinum* and *P. fimorum* were all present on the same sample of mouse dung pellets. No synnemata production by *P. robsamsonii* and *P. fimorum* was observed on the mouse dung pellets. It should, however, be noted that the pellets were dry at time of collection, and this might have prevented the production of synnemata on the mouse dung pellets.

Penicillium robsamsonii Frisvad & Houbraken, *sp. nov.* — MycoBank MB815872; Fig. 5

In: *Penicillium* subgenus *Penicillium* section *Robsamsonia.*

ITS barcode. KU904339 (alternative markers: *BenA* = KT698885; *CaM* = KT698894; *RPB2* = KT698904).

Etymology. The species is named after Robert A. Samson, celebrating his 70th birthday.

Type specimen. Denmark, Høve Strand, ex mouse dung, 2009, *J.C. Frisvad* (holotype CBS H-22341, cultures ex-type CBS 140573 = IBT 29466 = DTO 149-B6).

Diagnosis — Fasciculate colonies on CYA and MEA, pale brown reverse colour on CYA; stipes rough-walled; violet Ehrlich reaction.

Description — Colony diam, 7 d, in mm: CYA 17–22; CYA15°C 14–20; CYA30°C 13–19; CYA37°C no growth; MEA 18–24; YES 31–39; CYAS 13–18; creatine agar 11–17, moderate growth, no acid production absent, base formation present.
CYA, 25 °C: Colonies elevated at centre; sporulation strong; colony texture fasciculate; mycelium white; exudate present as large pale brown droplets; soluble pigment absent or weakly present, pale brown; radial sulcate, deep; margin entire to slightly irregular; conidia dull green; reverse pale brown. YES, 25 °C: Sporulation moderate to good, mycelium white; exudate present as small, hyaline droplets; soluble pigments present, brown; conidia dull green; reverse pale brown. MEA, 25 °C: Sporulation on MEA strong; colony texture floccose; mycelium white; exudate present, large droplets, brown; soluble pigments absent; conidia dull green; reverse brown in centre, edges not affecting reverse colour. DG18, 25 °C: Sporulation good to strong; colony texture slightly floccose in centre, velvety at the edge; mycelium white, conidia dull green; reverse pale brown in centre, edge pale. Ehrlich reaction violet.
Sclerotia absent. *Synnemata* absent. *Conidiophores* 100–200 µm long, with rough walled stipes, terverticillate, stipe 3–4 µm wide. *Metulae* (2–)3–5, 9.0–11 × 3.0–4.5 µm. *Rami* 1–3 per stipe, appressed, 10–18 × 3.0–4.5 µm. *Phialides* ampulliform to cylindrical with short necks, 3–7 per metula, 7.0–8.5(–9.5) × 2.0–3.0 µm. *Conidia* in long, distorted chains, smooth-walled, ellipsoid, (3.0–)3.5–4.5 × 2.5–3.5 µm.

Extrolites — andrastin E, chaetoglobosins, clavatols, a pyripyropen, quinolactacin, patulodin, roquefortine C.

Additional material examined. Denmark, Høve Strand, ex mouse dung, 2009, *J.C. Frisvad*, cultures IBT 29509 = CBS 140574 = DTO 149-B7.

Notes — *Penicillium robsamsonii* is phylogenetically closely related to *P. fimorum* (details on differences, see description of *P. fimorum*).

Penicillium samsonianum L. Wang, Frisvad, Hyang B. Lee & Houbraken, *sp. nov.* — MycoBank MB815873; Fig. 6

In: *Penicillium* subgenus *Penicillium* section *Osmophila.*

ITS barcode. KJ668590 (alternative markers: *BenA* = KJ668582; *CaM* = KJ668586; *RPB2* = KT698899).

Etymology. The species is named after Robert A. Samson, celebrating his 70th birthday.

Type specimen. China, Qinghai, Kekexili, N35°11'20" E93°07'28", 4578 m, ex grassland along the banks of Qumar River, no. HPJ58, 2013, *P-J. Han* (holotype HMAS 245107, cultures ex-type AS3.15403 = CBS 138919 = IBT 33392 = DTO 316-B7).

Diagnosis — *Penicillium samsonianum* is characterised by its good growth on CYA15°C (22–27 mm), poor growth and acid production on CREA, brown reverse on CYA, and the production of penitrem A, penitremone A, penitremone B, mycophenolic acid, patulin and roquefortine C.

Description — Colony diam, 7 d, in mm: CYA 20–26; CYA15°C 21–28; CYA30°C 5–12; CYA37°C no growth; MEA 16–23; YES 23–32; CYAS 20–27; creatine agar 7–15, poor growth, moderate acid production.
CYA, 25 °C: Colonies elevated in centre; sporulation moderate, mainly in centre; colony texture lanose; mycelium white, occasionally pale brown; exudate absent or present as large pale droplets; soluble pigments absent; radial sulcate; margin entire; conidia dull green, reverse brown. YES, 25 °C: Sporulation absent; mycelium white; exudate absent; soluble pigment production absent; reverse yellow. MEA, 25 °C: Sporulation variable; weak, moderate or good; colony texture lanose; mycelium white; exudate absent or present, large droplets, clear; soluble pigments absent; conidia bluish grey-green; reverse brown. DG18, 25 °C: Sporulation moderate to strong; colony texture floccose; mycelium white; conidia grey-green or grey to dull green; reverse pale or pale to pale yellow. Ehrlich reaction negative.
Sclerotia absent. *Conidiophores* arising from agar surface, (300–)400–600(–700) µm long, smooth-walled, terverticillate, occasionally bi- or quarterverticillate, stipe 3.5–4 µm wide. *Rami* 1–3 per stipe, (11–)14–18 × 3–3.5 µm. *Metulae* (2–)4–6 per ramus, (7–)9–14 × 2–2.5 µm. *Phialides* 4–6 per metula, ampulliform with distinguishable collula, 9–11 × 2–2.5 µm. *Conidia* born in short irregularly tangled chains, smooth-walled, globose, 3–3.5 µm.

Extrolites — Penitrem A, penitremone A, penitremone B, mycophenolic acid, patulin, roquefortine C.

Additional material examined. Canada, Saskatchewan, ex dog, *R.A.A. Morrall*, culture IBT 4175 = CBS 512.73 = DTO 327-D6. – Denmark, ex salami, *J.C. Frisvad*, culture IBT 15554 = CBS 316.97 = DTO 187-G1. – Italy, ex soil, 1960, *C.A. Ghillini*, culture IBT 16427 = CBS 343.61 = DTO 327-E2.

– Korea, ex. stems and leaves of *Viscum album* var. *coloratum*, *H.B. Lee*, cultures NIBR KOSPFG124291 = EML-WPF1 and NIBR KOSPFG124292 = EML-WPF2. – USA, Wyoming, DOE site; 11 km west of Rock Springs, ex A1 horizon soil; sagebrush (*Artemisia tridentata*), 1978, *M. Christensen*, culture IBT 13163 = RMF S89 = CBS 131220 = DTO 327-D7.

Notes — *Penicillium samsonianum* is phylogenetically most closely related to *P. osmophilum*. *Penicillium osmophilum* produces ascomata, and no ascomata or sclerotia were observed in *P. samsonianum*. Furthermore, *P. samsonianum* produces acid compounds on CREA and has a brown reverse on CYA, while *P. osmophilum* lacks acid production on CREA and the reverse colour on CYA is in shades of red-brown.

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